Marked-Up Version Showing Changes Made to the Claims

1. (Amended) An integration and expression plastid vector ~~competent~~ for stably transforming ~~the~~a plastid genome of a higher plant species where plant growth is inhibited by an antibiotic-free phytotoxic agent, wherein said integration and expression plastid vector is contained in ~~which comprises~~ an expression cassette which comprises as operably joined components, a 5' ~~part~~end of ~~the~~a plastid DNA sequence inclusive of a spacer ~~sequence~~region, a promoter operative in said plastid genome, a DNA sequence encoding a detoxifying enzyme ~~or protein~~ acting as a selectable marker, which is capable of detoxifying said ~~antibiotic-free~~ phytotoxic agent in ~~the cells~~a cell to the corresponding nontoxic compound, at least one restriction site for the insertion of a heterologous target ~~gene~~DNA sequence, a transcription termination region functional in said plastid, and ~~the~~ a 3' ~~part~~end of a plastid DNA sequence inclusive of the spacer sequence.

5. (Amended) ~~A~~The chloroplast vector of claim 2 wherein the molecule of interest is a polypeptide.

6. (Amended) ~~A~~The chloroplast vector of claim 3 ~~or claim 4~~, wherein said plastid is tobacco chloroplast.

8. (Amended) A vector of claim 4 ~~competent~~ for stably transforming the cholorplast genome where growth is inhibited by a phytotoxic aldehyde which is selected from the group consisting of acetaldehyde, formaldehyde, proprionaldehyde, butyraladehyde and betaine aldehyde

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9. (Amended) An integration and expression plastid vector ~~competent~~ for stably transforming ~~the~~a plastid genome of higher plant species where plant growth is inhibited by ~~a phytotoxic aldehyde~~betaine aldehyde wherein said integration and expression plastid vector comprises an expression cassette which comprises as operably joined components, a 5' ~~part~~end of ~~the~~a plastid DNA sequence inclusive of a 16S-23S spacer sequence, a promoter operative in said plastid genome, a DNA sequence encoding betaine aldehyde dehydrogenase (BADH) as a selectable marker which is capable of detoxifying said ~~phytotoxic~~betaine aldehyde in ~~the~~said cells to ~~glycine betaine~~the corresponding non-toxic compound, a heterologous DNA sequence which codes for a molecule of interest, a transcription termination region functional in said plastid genome, and a 3' ~~part~~end of a plastid DNA sequence inclusive of the spacer sequence.

10. (Amended) A stably transformed plant which comprises a chloroplast which has been stably transformed with ~~a~~the vector ~~of claim 8 or Claim 9,~~ or the progeny ~~thereof~~of the vector of Claim 9.

Cancel.

Cancel.

14. (Amended) ~~A~~The stably transformed plant of claim 10, wherein the plant is a monocotyledonous plant~~;~~ selected from the group consisting of rice, wheat, grass, rye, barley, oat ~~or~~and maize.

15. (Amended) ~~A~~The stably transformed plant of claim 10, wherein the plant is a dicotyledonous plant~~;~~ selected from the group consisting of soybean, peanut, grape, sweet potato, pea, canola, tobacco, tomato ~~or~~and cotton.

16. (Amended) ~~A stable~~The stably transformed plant of claim 10, wherein the plant is a tobacco, tomato, potato, rice, brassica, cotton, maize or soybean plant.

17. (Amended) ~~A stable~~The stably transformed plant of claim 10, wherein the plant is a homoplasmic plant.

18. (Amended) ~~A~~The vector of any one of claims ~~2-9~~2-3, 5, 7 or 9, wherein the selectable marker is driven by a promoter in green and non-green tissues selected from the group consisting of the 16SrRNA promoter, the psbA promoter, the alpB promoter, or the accD promoter.

19. (Amended) A method for ~~transforming the~~introducing into plastid genome of a plant cell a DNA sequence encoding for detoxifying enzyme, which method does not require selection for successful transformants by the detection of antibiotic resistance, said method comprising introducing into cells of a plant species whose growth is inhibited by an antibiotic-free phytotoxic agent, an expression cassette which comprises as operably linked components, a 5' ~~part~~end of a plastid DNA sequence inclusive of a spacer sequence, a promoter operative in said plastid, a DNA sequence encoding a detoxifying enzyme ~~or protein~~ acting as a selectable marker for transgenic plant cells and capable of detoxifying said phytotoxic agent in the cells to the corresponding nontoxic compound, a heterologous

target DNA sequence, a transcription termination region functional in said plant chloroplast cells, and the 3' ~~part~~end of the plastid DNA sequence inclusive of a spacer sequence.

20. (Amended) The method of claim ~~18~~19 wherein the heterologous target DNA sequence codes for a molecule of interest.

21. (Amended) The method of claim ~~18~~19 wherein the ~~antibiotic-free phytotoxic agent~~plastid DNA sequence codes for a phytotoxic aldehyde and the detoxifying enzyme or protein is a aldehyde dehydrogenase capable of detoxifying said phytotoxic aldehyde.

22. (Amended) The method of claim ~~18~~19 wherein the phytotoxic aldehyde ~~which~~ is selected from the group consisting of acetaldehyde, formaldehyde, ~~propronaldehyde~~proprionaldehyde, ~~bytyraldehyde~~butyraldehyde and betaine aldehyde.

23. (Amended) A method of claim ~~18~~19, wherein said method further comprises culturing said plant in a plant growth medium comprising said phytotoxic aldehyde, and selecting ~~transformed~~a plant ~~cells~~cell that has had the DNA encoding sequence for a detoxifying enzyme introduced and hence is capable of growth in the presence of said phytotoxic aldehyde.

24. (Amended) ~~A~~The method of claim ~~22~~23, wherein said method further comprises regenerating a transformed plant from said transformed plant cells.

25. (Amended) ~~A~~The method of claim ~~20~~21 wherein said phytotoxic aldehyde and the aldehyde dehyrodgenase is ;betaine aldehyde dehydrogenase (BADH).

26. (Amended) ~~A~~The method of claim ~~24~~25, wherein said DNA sequence encoding a detoxifying enzyme is from ~~plants such as~~ sugar beet~~;~~ or spinach plants.

27. (Amended) ~~A~~The method of claim ~~24~~25, wherein said DNA sequence is from a microorganism, ~~such as~~ E.~~Coli~~coli.

28. (Amended) ~~A~~The method of claim ~~18~~19, wherein the promoter is selected from ~~a~~the group consisting of 16SrRNA, psbA, accD and alpB ~~promoters~~.

29. Cancel.

30. (Amended) ~~A~~The method of any one of claims ~~18-27~~19-28, where the expression cassette further comprises a ribosome binding site (rbs) and a 5' untranslated region ~~(5'UTR)~~5' UTR to enhance expression.

Please add the following new claim:

31. (New) An integration and expression plastid vector competent for stably transforming the tobacco plastid genome where growth is inhibited by betaine aldehyde, a phytotoxic aldehyde, which comprises an expression cassette which comprises as operably joined components, a 5' part of the plastid DNA sequence inclusive of a spacer sequence, a promoter operative in said plastid, a DNA sequence encoding spinach betaine aldehyde dehydrogenase (BADH) as a selectable marker which is capable of detoxifying said phytotoxic aldehyde in the cells to glycine betaine, a heterologous DNA sequence which codes for a molecule of interest, a transcription termination region functional in said tobacco plastid, and a 3' part of a plastid DNA sequence inclusive of the spacer sequence.

Please cancel Claims 12, 13, and 28 (renumbered 29) without prejudice.

Marked-Up Version Showing Changes Made to the Abstract

Please add the abstract as follows:

An integration and expression plastid vector competent for stably transforming a plastid genome of higher plant species where plant growth is inhibited by an antibiotic-free phytotoxic agent, wherein said integration and expression plastid vector is contained in an expression cassette which comprises as operably joined components, a 5' end of a plastid DNA sequence inclusive of a spacer region, a promoter operative in said plastid genome, a DNA sequence encoding a detoxifying enzyme acting as a selectable marker, which is capable of detoxifying said phytotoxic agent in a cell to the corresponding nontoxic compound, at least one restriction site for the insertion of a heterologous target DNA sequence, a transcription termination region functional in said plastid, and the 3' end of a plastid DNA sequence inclusive of the spacer sequence.

REMARKS

Applicant acknowledges the Examiner's rejection of Claims 1-12, 14-17, 19-28, and 30. Applicant further acknowledges the objection to Claims 13, 18 and 29. Applicant has canceled Claims 12-13 and 29, and added new Claim 31 based on pages 10, and 12-13 of the Specification. No new matter has been added. Thus Claims 1, 11, and 14-30 are now pending in the application and Applicant asks for consideration of new Claim 31.

Applicant appreciates the Examiner's helpful suggestion in pointing out the numbering problem. Accordingly the Claims have been renumbered. The application, as filed, included an Abstract on a separate sheet as page 29. Further, the published PCT application included an abstract. Copies of both abstracts are enclosed. Applicant has also submitted, on a separate sheet, an abstract of the disclosure as required by 37 CFR 1.72(b) which is requested to be entered if the original cannot be found. Finally, the Applicant has corrected the priority in this case. The filing date of the provisional application was correct. However, the number of the application has been corrected to read "60/209,762" in the first paragraph of the Specification.

Turning now to the claim objections, Claim 13 and 29 have been canceled, and Claim 18 has been amended to eliminate multiple multiple dependencies. Accordingly, Applicant requests that Claim 18 be treated on its merits. At the helpful suggestion of the Examiner the Applicant has added a proper article at the start of claims that remain under consideration, along with correcting other minor defects that were pointed out in the Examiner's objection.

Response to §112 Rejections

In response to the rejections based on 35 U.S.C. § 112, 1st paragraph, the Applicant respectfully submits that as a result of the amendments to the claims, and also for the reasons set forth below in detail, this rejection is obviated.

The Applicant respectfully submits that the specification , as written, enables one skilled in the art to use Applicant's novel selection system in a variety of plants by interchanging a variety of aldehyde dehydrogenases capable of detoxifying a variety of corresponding phytotoxic aldehydes. The prevailing standard for determining whether the specification meets the enablement requirement is whether the experimentation needed to practice the invention is undue or unreasonable. *See* Mineral Separation v. Hyde, 242 U.S. 261, 270 (1916). As long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the scope of the claim, then the enablement requirement of 35 U.S.C. §112 is satisfied. *In re* Wands, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988).

According to the aforementioned standard for determining enablement, Applicant respectfully submits that the specification provides enablement for a plastid transformation vector containing a gene coding for BADH and other aldehyde dehydrogenases as well as methods for transforming any of a number of plant species. At the time the Applicant filed this specification, there were known sequences coding for aldehyde dehydrogenase detoxifing enzymes other than BADH. See U.S. Patent 5,633,153 to Ursin, already of record in this case.

The Applicant has incorporated by reference a universal vector capable of transforming the plastid genome of different plant species (page 9, lines 17-21). For the Examiner's conveince we have included non-limiting extracts, which illustrate that the Applicant was in possession of a universal chloroplast vector.

> The invention provides universal chloroplast integration and expression vectors which are competent to stably transform and integrate genes of interest into chloroplast genome of multiple species of plants. Transformed plants and their progengy are provided. Monocotyledonous and dicotylendonous plants are transformed which have never been transformed heretofore.
>
> It has been discovered contrary to the conventional belief, that the chloroplast (ct) genome of plants contains spacer regions with highly conserved nucleotide sequences. The highly conserved nature of the nucleotide sequences of these spacer regions of the chloroplast genome makes such spacer regions, it has been discovered, ideal for the construction of vectors to transform chloroplasts of widely varying species of plant, this without the necessity of constructing individual vectors for different plants or individual crop species, which would require first a determination of the DNA sequence of each of the chloroplast genomes.
>
> **The Universal Vector.** The invention has several useful embodiments. The invention provides a universal integration and expression vector hereinafter referred to as "UV" and its use for the expression of at least one phenotype in a variety of different plants.
>
> The integration expression universal vector of the invention comprises an expression cassette (further described below) which comprises the necessary genetic elements to transiently or preferably stably transform the plastids e.g. chloroplast genome of a target plant cell with a foreign (heterologous) DNA coding for a molecule of interest, like a phenotype to be expressed by the plant or a non-plant high value molecule, like a biologically active peptide (or polypeptide). The universal vector is constructed with a transcriptionally active region of a chloroplast genome that is highly conserved in a broad range of chloroplast genomes of higher plants. Preferably that region is the spacer 2 region; the intergenic spacer region between the t-RNA^{Ile} and the tRNA^{Ala} region. Such region is often referred to herein as a "spacer" region because in the chloroplast genome it is intergenic between several genes in the

rRNA operon which is transcribed by one promoter. When built into the universal vector such region is generally referred to herein as a "border" or preferably as a "flanking sequence" or "flanking sequences". This is because in the universal vector, the operably joined genetic elements for transforming stably the plastid of the target plant are flanked on each side by a sequence i.e. a fragment of the spacer region. The flanking sequences in the vector and the spacer sequences in the chloroplast genome have sufficient homology to each other to undergo homologous recombination. The universal vector is inserted into the spacer of a transcriptionally active region in the chloroplast genome. Generally, the spacer region is positioned in the inverted repeat region of the chloroplast genome. The rest of the construct, ie. other than the flanking sequences and the expression cassette, is generally referred to herein as the "vector" which comprises bacterial sequences, like the plasmid cloning vectors pUC, pBR322, pGEM or pBlueScript.

Furthermore, the specification at pages 12-13 provides extensive instructions for identifying an appropriate manner to introduce a transformation vector. Heifetz (2000, Biochimie 82: 655-666) teaches that complete plastid genomes have been sequenced from a variety of plant and algal species providing a wealth of information regarding conservation of reading frames and regulatory sequences. Plastid-encoded messages have also been found to be regulated post-transcriptionally over an unusually broad range [8-10]. Taken together, these factors mean that heterologous genes or operons can be inserted into the plastid genome in a site-specific manner and can be expressed at levels ranging from low to extremely high, or alternatively the coding sequences of endogenous plastid genes can be mutated in a directed fashion by virtue of the high efficiency of plastid homologous recombination. This is particularly true in light of the publication of the complete DNA sequence and genomic maps of at least fourteen different plant species. The following

complete chloroplast genome sequences were already available in Genbank at the time of filing of this application:

Marchantia polymorpha 121,024 kbp

Nicotiana tabacum 155,844 kbp

Oryza sativa 134,525 kbp

Epifagus virginiana 70,028 kbp

Pinus thunbergii 119,707 kbp

Zea mays 140,387 kbp

Arbidopsis thaliana 154,478 kbp

Triticum aestivum 134,540

Euglena gracilis 143,172 kbp

Cyanophora paradoxa 135,599 kbp

Odontella sinensis 119,704 kbp

Porphyra purpurea 191,028 kbp

Chlorella vulgatis 150,613 kbp

Mesostigma viride 118,360 kbp

Thus one could, if so inclined, simply search appropriate spacer regions of the various plastid genomes. A number of conserved intergenic spacer regions, among various plant species, are well known in the art. Sugita et al., "Regulation of Gene Expression in

Chloroplasts of Higher Plants," *Plant Molecular Biology*, vol. 32, pp. 315-326, (1996). In addition, numerous incomplete chloroplast genome sequences are also available in the Genbank with more being added all the time, without undue experimentation.

Applicant kindly asks the Examiner to consider Example 18 from the "Revised Interim Written Description Guidelines Training Materials" (copy enclosed), where it is pointed out that "one skilled in the art would recognize that Applicant was in possession of all the various expression methods necessary to practice the claimed invention."

Example 18, a method for producing proteins, correlates with the Applicant's claims relating to selecting genetically engineered plants without the use of antibiotics as a selectable marker. In Example 18 the "*Neurospora crassa* mitochondrial gene expression is essential to the function/operation of the claimed invention." Similarly, chloroplast expression of a selectable marker, which is detoxifying enzyme, is essential to the function/operation of the Applicant's claimed invention. Example 18 is drawn to an allowed genus despite the fact that there was only a single embodiment reduced to practice. In Example 18 there was only the expression of E-galactosidase. The mitochondria only expressed one protein with the help of one integration vector. Applicant's invention uses the universal vector PLD-BADH to transform tobacco plastid with BADH where the BADH functions as a selectable marker in the tobacco plastids. In other words, Applicant's claim is drawn to a genus, but the single embodiment is representative of the genus based on a selectable marker expression system.

Applying the reasoning of Example 18 to the Applicant's claims, any of a variety of detoxifying enzymes in the Specification, could be inserted into a tobacco plastid via the universal vector and then consequently tested for expression with a corresponding phytotoxic aldehyde. One skilled in the art would recognize that the Applicant was in possession of all the various expression methods necessary to practice the Applicant's invention.

Turning now to the Examiner's assertion that Applicant has failed to disclose vectors besides the PLD-BADH vector, we ask the Examiner to consider page 9, lines 11-17 of Applicant's specification, Fig. 9A and 9B of the Applicant's specification, and the references that have been incorporated by reference. The aforementioned, teach a variety of transformation vectors capable of integrating detoxifying enzymes.

Response to §112, 2nd Paragraph Rejections

Applicant has amended the claims to provide the proper antecedent basis, correct Markush format and omnibus clams, and clear up any indefiniteness of the claims. Further, Applicant has amended the method claims in order to avoid omitting essential method steps. As a result of these amendments, Applicant submits the claims are no longer indefinite and, hence, are now in a condition for allowance.

One of the objections to 1 and 19 pertains to the use of the phrase "protein acting as a selectable marker," and how this would occur. As set forth in the specification, betaine aldehyde dehydrogenase is an enzyme which detoxifies betaine aldehyde. This can be used

as a selectable marker by growing transformed plants and cells in the presence of an otherwise phytotoxic amount of betaine aldehyde. Transformed plants and cells survive due to the presence of the enzyme. Enzymes are proteins which catalyze chemical reactions, such as the detoxifying dehydrogenation of betaine aldehyde. BADH is one such enzyme/protein.

Response to §103 Rejections

Applicant respectfully submits that solicited Claims 1-3, 5-7, 9-11, 14-21, and 23-30 are patentable over Maliga et al. (1999, U.S. Patent 5,877,402) in view of Rathinasabapathi et al. (1994, Planta 193:155-162). Applicant respectfully submits Maliga et al. teaches away from the use of genes encoding aldehyde dehydrogenases as selectable markers, as is suggested by Rathinasabapathi et al. Maliga et al. teach a *non-lethal* selectable marker gene which confers a selectable phenotype to cells containing plastids transformed with the DNA construct (Col. 4, lines 25-27). The Applicant has demonstrated that non-lethal selection was not required for chloroplast transformation (page 6, lines 3-7).

Maliga et al. points out that selection markers have been identified by screening culture plant cells for *mutants* resistant to various substances, most notably, antibiotics and herbicides (Col. 3, lines 13-16). Furthermore, Maliga et al. teaches a chloroplast transformation system whose transformation process is performed using non-lethal selectable markers. Therefore, one skilled in the art would be led to believe that any derivative of Maliga et al's chloroplast transportation system would only function if using

non-lethal selectable markers. It is well-known in the art that accumulation of betaine aldehyde is lethal to plant cells, and therefore, one skilled in the art, upon reading Maliga, would believe non-lethal selection was required for chloroplast transformation.

We invite the Examiner's attention to all of Maliga et al.'s Examples 1-7, which all use a non-lethal selectable marker derived from *Nicotiana tabacum* and *Nitcotiana plumbaginifolia*.

One skilled in the art, after reviewing the teachings of Maliga et al., would be led away from using BADH as a selectable marker, because Maliga et al. teaches using *Nicotiana* mutants to identify <u>non-lethal</u> selectable markers. There is no suggestion that a selectable marker, not identified by *mutant* resistance, could integrate into a plastid genome via the expression vectors disclosed in Maliga et al. Maliga et al. requires that the expressed DNA is positioned relative to the selectable marker gene so as not to interfere with its ability to confer the non-lethal selectable phenotype to cells containing transformed plastids (Col. 5, lies 18-24). This implies that the selectable marker/expressible DNA relationship is sensitive. One skilled in the art would reasonably conclude that inserting BADH as a selectable marker, in place of a traditional antibiotic, may alter the expression of the expressible DNA. Therefore, Maliga et al. does not provide motivation to use a non-mutated BADH gene obtained from either spinach or sugar beet genomes as use as a selectable marker.

Furthermore, it was not obvious to transform plastids using BADH as a selectable marker with the transformation vectors taught by Maliga et al. While it was known that

BADH was a plant enzyme, it could not be conclusively demonstrated that this was a chloroplast-generated enzyme. BADH lacks the typical transit peptide found in all chloroplast proteins imparted from the cytosol. The absence of these typical transit peptides would leave one skilled in the art questioning whether the proper cleavage of BADH enzyme in the stroma within plastids could be fully functional. One skilled in the art could only speculate whether the BADH enzyme would be catalytically active, without proper cleavage, within plastids. Thus one skilled in the art could nor introduce the gene for BADH into a plastid <u>with the expectation</u> that BADH would be produced, and function to detoxify betaine aldehyde. Logically, it follows that if the BADH is not catalytically active within the plastid, it could not serve as a selectable marker. We invite the Examiner's attention to *In Re Lalu*, 747 F.2d 703, 223 U.S.P.Q.1257 (Fed. Cir. 1984) which held that there is no motivation to substitute a claimed compound for a prior art compound, unless the two compounds have a *common utility*. *In Re Lalu* is analogous to the current situation because there is no suggestion that the selectable markers exemplified in Maliga et al. share any common utility, structure or origin with the BADH selectable marker.

At the very most it was only obvious to try utilizing BADH as a selectable marker in the plastid transformation vectors disclosed in Maliga et al. Prior to the Applicant's invention no plastid genome had been modified without the use of antibiotic selection markers. Rathinasabapathi et al. merely pointed out that the use of BADH as a selectable marker was of interest. Rathinasabapathi et al. observed that the BADH *enzyme*, not the gene, is targeted to the chloroplasts. Rathinasabapathi et al. does not teach that BADH can

be stably integrated into the plastid genome to produce a functional enzyme. Applicant has discovered that the BADH gene can be integrated into the chloroplast gene.

> The strategy to distinguish between nuclear and chloroplast transgenic plants was to land one primer (3P) on the native chloroplast genome adjacent to the point of integration and the second primer (3M) on the aadA gene (Figure 1). This primer set generated 1.6 kb PCR product in chloroplast transformants (Figure 4). Because this product cannot be obtained in nuclear transgenic plants, the possibility of nuclear integration can be eliminated.

Without knowledge that the BADH gene was integrated into the chloroplast genome, rather than simply expressed in it, one skilled in the art would have no expectation that Maliga et al's chloroplast transformation vector and methods could successfully integrate BADH into the chloroplast genome of plants. The importance of chloroplast *gene* integration has been articulated in the specification.

> Plastid genetic engineering, particularly chloroplast genetic engineering, is emerging as an alternative new technology to overcome some of the environmental concerns of nuclear genetic engineering (reviewed by Bogorad, 2000). One common environmental concern is the escape of foreign gene through pollen or seed dispersal from transgenic crop plants to their weedy relatives creating super weeds or causing genetic pollution among other crops (Daniell 1999B). Keeler et al. (1996) have summarized valuable data on the weedy wild relatives of sixty important crop plants and potential hybridization between crops and wild relatives. Among sixty crops, only eleven do not have congeners and the rest of the crops have wild relatives somewhere in the world. In addition, genetic pollution among crops has resulted in several lawsuits and shrunk the European market of Canadian organic farmers (Hoyle 1999). Several major food corporations have required segregation of native crops from those "polluted" with transgenes.

In fact, Rathinasabapathi et al. performed their experiment using antibiotic (specifically kanamycin) resistance, as selection markers (Col. 1, page 157).

Rathinasabapathi et al. used five BADH construction vectors, which exhibited significant variation in BADH expression among the constructs (left column, page 157). This illustrates that one skilled in the art would not find it obvious that BADH would be consistently expressed through the transformation vectors disclosed in Maliga et al.

Solicited Claims 1-3, 5-7, 9-11, 14-21 and 23-30 have been rejected under 35 U.S.C. §103 based on Maliga et al. in view of Ursin (1997, U.S. Patent 5,633,153). Applicant respectfully submits that the aforementioned claims as amended are patentable over Maliga et al. in view of Ursin.

Applicant respectfully submits that Ursin teaches a method of using an aldehyde dehydrogenase as a selectable maker for *nuclear* transgenic plants. Ursin discloses a DNA construct coding for an aldehyde dehydrogenase through eukaryotic promoters used for *nuclear* transformation and culturing such transformed cells in growth media containing the corresponding phytotoxic aldehyde; the transformed plants demonstrate resistance to the phytotoxic aldehyde. In fact, Ursin states "the manners in which the ALDH constructs are introduced into the host plant cell is NOT critical to this invention." (emphasis added) Hence, Ursin teaches one skilled in the art that how and where BADH is integrated and expressed is irrelevant. Applicant's invention, however, has shown not only where BADH is expressed, but also, that it is of great significance that BADH is expressed in plastids rather than the nucleus. Nuclear BDAH, having a high GC content could not be expected to express well in AT-rich prokaryotic plastid compartments, because the codon usage is very different between the prokaryotic chloroplast compartment and the eukaryotic nuclear

compartment. Therefore, it was not obvious to express a nuclear gene in a plastid compartment taught in Maliga et al.

We further invite the Examiner's attention to Example 2 of Ursin et al., which discloses constructs which are structurally dissimilar to Maliga et al. Ursin teaches that "there are several approaches that could be used to provide for localization of the BADH protein in the cytoplasm. For example, a BADH gene from an organism which does not require *plastid* targeting mechanisms could be used" (Col. 5, lines 32-35). This leads one skilled in the art to believe that BADH cannot be targeted to plastids, which teaches directly away from Applicant's claimed invention.

Applicant respectfully submits that solicited Claims 1-3, 5-7, 9-11, 14-21 and 23-30 are patentable over Maliga et al. in view of Holmstrom et al. (1994, Plant J. 6: 749-758).

Holmstrom et al. teaches directing the bacterial BADH, BetB, with a transit peptide into the chloroplast of *N.tabacum*. Holmstrom et al. fails to teach a spinach or sugar beet BADH gene direct to plant chloroplast in the *absence* of a typical transit peptide. As with Rathinasabapathi et al., Holmstrom et al. merely indicates that BetB protein was incorporated into and processed inside the chloroplast. Applicant, however, has discovered that BADH is actually transcribed and expressed in chloroplasts. As was explained in the specification, there are a number of benefits to having chloroplasts transformed with a DNA sequence as opposed to nuclear DNA transformants. One skilled in the art would be discouraged from attempting to use BADH genes of plant origin in chloroplasts via Maliga et al's expression vectors. Holmstrom et al. states that "*E.coli* betaine biosynthetic enzymes

appear to be more related to the corresponding mammalian enzymes than to plant ones" (Left Col. Page 750). This disclosure discourages one skilled in the art from attempting to insert plant BADH genes into chloroplasts.

In light of the foregoing, Applicant respectfully submits that Claims 1-3, 5-7, 9-11, 14-21 and 23-30 are patentable over Maliga et al. in view of Holmstrom et al. and further respectfully submits that the claims are now in complete condition for allowance, which is respectfully requested.

Respectfully submitted,

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